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Captive management and breeding of Romer's tree frog *Chirixalus romeri*

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In the early 1990s, a significant portion of the range of the endemic Romer's tree frog *Chirixalus romeri* was threatened by the construction of the new international airport in Hong Kong. Melbourne Zoo, Australia, partnered with The University of Hong Kong to establish a captive-breeding programme as part of a broader conservation initiative to secure the species in the wild. Large numbers of frogs were successfully bred at both facilities, underpinning the success of subsequent releases.

Key-words: captive breeding; conservation partnerships; growth; management; Romer's tree frog.

INTRODUCTION

The rhacophorid genus *Philautus* comprises many species found in tropical and subtropical Asia (Inger, 1999; Zhao, 1999; Frost, 2004). Romer's tree frog *Philautus romeri* was discovered in 1952 living in a small, remote cave by the sea, on Lamma Island, Hong Kong (Smith, 1953). It was then observed a year later, but was subsequently not seen again until it was rediscovered in 1984. In 2001, it was tentatively placed in another genus, *Chirixalus*, based on its having a free tadpole stage, which members of the *Philautus* genus do not have (Bossuyt & Dubois, 2001).

The maximum adult length of *Chirixalus romeri* is 20 mm (Plate 1). The body is tan to brown above, with a darker brown or black X-shaped marking on the back, made up of two lines that do not always meet in the centre (Karsen *et al*., 1998). Although referred to as a tree frog, it is usually found at ground level or sometimes in low bushes.

In 1991, *C. romeri* was only known from three Hong Kong islands: Chek Lap Kok, Lantau and Lamma (Karsen *et al*., 1998; M. Lau, pers. obs). Subsequent extensive field surveys throughout Hong Kong confirmed the frog's presence on these three islands, plus Po Toi Island in Hong Kong (Lau, 1992, 1998).

Concerns over the frog's status were raised in 1991 by the World Wide Fund for Nature (Hong Kong) and other conservation bodies, arising from plans by the Hong Kong Government to build a new international airport on Chek Lap Kok Island, with associated construction also having a significant impact on *C. romeri* breeding sites on neighbouring Lantau Island. Chek Lap Kok Island originally covered an area of *c.* 5 km^2 and the airport development required its expansion to about 8 km^2 of airport, with bridges joining Chek Lap Kok Island to the neighbouring island of Lantau. A road and rail link was also to be constructed along Lantau's northern coast first to Tsing Yi Island and then to the New Territories. A new town was to be built on northern Lantau, resulting in the loss of *C. romeri* breeding sites in that area. The concerns were communicated to the IUCN/SSC Conservation Breeding Specialist Group, which called for support from the world's zoos in implementing two key recommendations in the Environmental Impact Assessment for the project: (1) to establish secure off-shore captive populations; (2) to undertake studies of *C. romeri* ecology and identify suitable re-release sites.

Plate 1. Romer's tree frog *Chirixalus romeri***.** *Michael Lau***.**

Melbourne Zoo (MZ), Australia, agreed to support the project in October 1991, focusing on establishing a secure offshore captive population at the Zoo. The population would be held until such time as appropriate release sites had been identified and then the frogs would be returned to Hong Kong. (Zoo Berlin also agreed to participate in the conservation-breeding programme and received 30 founder individuals. Unfortunately, all these frogs died in the summer of 1994.)

Funds were subsequently provided by the Royal Hong Kong Jockey Club Charities Ltd (RHKJCCL) to enable ecological studies and a translocation programme to be undertaken for the species by The University of Hong Kong (HKU) from 1991 to 1997.

The species was not identified as threatened when this project commenced, but has since been listed under the Hong Kong Wild Animals Protection Ordinance (Chapter 170) and as Endangered by the IUCN (The World Conservation Union) (Karsen *et al*., 1998; IUCN, 2006).

METHODS

Collection

Collection of *C. romeri* from Chek Lap Kok Island by HKU researchers began in November 1991 and ended in December 1992, just before the last patch of habitat was cleared for airport construction. The remaining habitats on the island were visited at least once every month during this period. A visit in April 1992 included staff from MZ and resulted in 30 frogs being captured and transferred to MZ 4 days later.

Searching for *C. romeri* was undertaken in all appropriate microhabitats during the day and at night, although most frogs were found at breeding sites at night. Numbers were recorded and adults were sexed, with the presence of vocal sacs and smaller size of ♂♂ used to distinguish the genders. Although Smith (1953) stated that ♂♂ of *C. romeri* do not possess secondary sexual characteristics, ♂♂ collected in this study had white nuptial pads on the first two fingers and a single external subgular vocal sac. However, the nuptial pads were not easily apparent to the naked eye and were not used as a diagnostic feature in sex determination.

Males started calling soon after dark and were located using headlamps by slowly scanning along the edges of shallow pools. Most calling ♂♂ were not collected initially, on the premise that they would entice the ♀♀ out. Many more ♂♂ were encountered than ♀♀ and all ♀♀ encountered were collected. From October to December 1992, all frogs found were collected, as it was approaching the end of the rescue operation. Egg clutches were collected in the early visits but this practice was discontinued because most eggs did not survive the long journey back to HKU.

Captive management and breeding

Housing Adults at HKU and MZ were maintained in acrylic terrariums (60 cm × 40 cm × 40 cm high at HKU and 61 cm × 31 cm × 31 cm high at MZ) with lids lined with plastic mosquito netting. The substrate was local soil (2–5 cm deep) with a top layer of leaf litter (Plate 2), which was changed every 6 months. A container (35 cm × 25 cm at HKU and 15 cm diameter at MZ) of shallow aged tap water (up to 3 cm deep) was placed in each tank for egg laying. Tap water was allowed to stand for 24–48 hours before use. A few dead leaves, twigs and stones were

Plate 2. The diminutive Romer's tree frog *Chirixalus romeri* **in leaf litter.** *Michael Lau***.**

placed in the water tray on which ♀♀ could attach their eggs. The terrariums were sprayed with water daily to increase the humidity. Up to 25 adults (more ♂♂ than ♀♀) were housed in each terrarium at HKU, while ten adults were housed in each container at MZ.

At HKU, the terrariums were kept in air-conditioned rooms illuminated by 40 W fluorescent tubes connected to a timer set with a 12 hour daylight cycle. Air temperature in the rooms varied from 20–29 °C in summer to 16–25 °C in the winter, while water temperature fluctuated from 19–27 °C in summer to 15–25 °C in winter. At MZ, two of the tanks were placed on a heated, off-exhibit table in the Zoo's Reptile House at 20–27 °C. The third tank was placed in an adjacent off-exhibit area that could be viewed by Zoo visitors. Ambient light was provided through sky-lights, supplemented by 40 W fluorescent tubes during the day.

Between August and November 1992, two modifications were made to the management regime at MZ to address health and hygiene issues that had become apparent over the preceding 6 months:

1. Sylvania® or NEC® 40 W black-light fluorescent tubes (BLB 40) were placed 30 cm above the holding tanks, to correct curvature of the frogs' femurs, which was considered to have arisen owing to a vitamin D deficiency caused by inadequate exposure to ultraviolet light. The lights were on timers for 30 minutes each morning and 30 minutes each afternoon. Application of the ultraviolet lights at MZ corrected the problem of bone curvature in the frogs.

2. The depth of the substrate was increased to 9–10 cm. The composition was also changed to a 4–5 cm-deep layer of palm peat on top of a 5–6 cm layer of aquarium pea gravel. All this rested on a piece of fine wiremesh and plastic egg-crate mesh. Below this, at the very bottom of each tank, was a 1–3 cm space where leached water and toxins were drained away through drainage holes in the base of each tank. The deeper substrate allowed for higher humidity to be maintained owing to decreased moisture loss. It also accommodated the limited Zoo staff resources by reducing or minimizing servicing of the increasing number of holding tanks. The addition of small grass tussocks allowed the frogs the opportunity to get off the tank floors. Frog wastes were also leached away by daily spraying. Anaerobic bacteria utilize nitrites and nitrates as they are leached through substrates and, hence, the deeper substrates acted as biological filters by converting organic compounds into organic wastes.

These two modifications were adopted at HKU in July 1992.

Diet Adult frogs at HKU and MZ were fed a staple diet of artificially raised young crickets *Teleogryllus oceanus* and wingless fruit flies *Drosophila melanogaster* once every 2 days. These were dusted weekly with Tetra vitamin mix before being offered to the frogs. This diet was supplemented at HKU with termites locally collected at least once every month.

Reproduction Terrariums were checked daily at both institutions. When eggs were found, the water tray was removed and replaced by a new tray. Eggs were checked daily and any unfertilized/dead eggs were removed. Tadpoles were given boiled iceberg lettuce or endive supplemented with high-protein fish food (Sanyu) once a week when they started feeding. The tadpoles were raised in trays and small plastic tanks (20 cm × 12 cm × 8 cm deep) in 20 mm of aged tap water at a density of less than six per 100

cm^2. Several leaves were provided for cover and the water was changed daily. When the hind limbs had developed (stage 38 onwards; Gosner, 1960), fish-food applications were increased to twice a week. At this stage, the containers were raised at one end to provide a dry area for the metamorphosed froglets.

Newly metamorphosed froglets at HKU were transferred to terrariums that were similar to those used for breeding adults, except that water trays were not provided. They were fed daily with very small leaf-litter arthropods (mainly *Collembola* and mites) until they were developed enough to cope with the adult diet. The small arthropods were either extracted from freshly collected forest leaf litter placed in a Berlese–Tullgren funnel, or were attracted to cut potatoes placed on damp soil and leaf litter. Metamorphlings at MZ were initially set up in 30 cm × 21 cm × 10 cm-deep plastic containers (*c*. 7 litres) and transferred to larger mesh-topped plastic tanks (23 litres) at about 1 month of age. The tanks were landscaped in a manner similar to the adults' tanks, but the substrate was palm peat. The young frogs fed readily on fruit flies and small grass flies.

Growth At HKU, clutch size, the number of unfertilized eggs, egg mortality and hatching time were determined when the eggs found in the breeding tanks were obviously from a single ♀. Tadpole mortality and time to reach metamorphosis were also determined. Several groups of froglets were raised in separate tanks to sexual maturity, and the sex ratio and mortality rate of juveniles were monitored. A group of adults [8.5 (♂.♀)] metamorphosed from tadpoles collected near Fu Tau Shan, Chek Lap Kok Island, in July 1991, were kept in one tank until June 1996. Data on the number of clutches of eggs produced per year, mortality rate and longevity were obtained from this group.

RESULTS

Collection

The species did not appear to have particularly strict environmental requirements for reproduction, as adults were found in or close to natural pools, man-made earthen excavations, concrete gutters and the abandoned trappings of village life. Tadpoles and spawn were found in natural pools, as well as in abandoned ceramic pots, filing-cabinet drawers and a refrigerator. The common features of these sites, however, were overhanging vegetation, still or very slowly flowing water and a thick base layer of decaying vegetation.

When the operation began in November 1991, the breeding season was over and no individuals were observed for 4 months. Many more *C. romeri* were observed during the breeding season between March and August 1992. In November 1992, many individuals were discovered at two damp localities: (1) a seep, surrounding plantation, abandoned fields and forest at Kwo Lo Wan (65 adults and seven juveniles); (2) a stream and riparian forest at Fu Tei Wan (11 adults and one juvenile). In a subsequent visit to these two areas in December 1992, 15 adults and one juvenile were found at the Kwo Lo Wan site and none at the Fu Tei Wan site. This suggested that most *C. romeri* were collected, at least in these two areas. It was also noted that the *C. romeri* found in November and December 1992 were not hiding, indicating that this species did not hibernate during the cool, dry part of the year. A total of 220 adults (150 ♂♂ and 70 ♀♀), nine juveniles, four metamorphlings, eight tadpoles and seven egg clutches was collected and transferred to HKU.

Captive management and reproduction

HKU At least 188 clutches of eggs were obtained from the Chek Lap Kok *C. romeri* kept at the HKU between March 1992 and June 1996. Of these, 108 clutches were laid by ♀♀ collected from Chek Lap Kok Island, either as adults or tadpoles, 76 clutches were produced by captive-bred F1 adults, and four clutches were obtained from frogs sent back to HKU from MZ.

At HKU, ♂ *C. romeri* usually called at night. Amplexing pairs were seen only at

night and the amplectic position was invariably axillary. The reproductive behaviour of captive animals matched that of wild individuals. Eggs were laid every month although there were far more clutches produced from February to August than between September to January. The clutch size, fertilization rate, hatching time, average larval periods in a clutch and mortality rates of eggs and larvae observed in captivity are shown in Table 1.

Juvenile (i.e. froglet) mortality and sex ratio was monitored in five groups of captive *C. romeri*. The mean juvenile mortality rate was 49% (range 31–71%) and the sex ratio was 1:0 · 69 (range 1:0 · 60–1:0 · 94).

The longevity, mortality and number of reproductive attempts of a group of 8.5 frogs that metamorphosed in August 1991 and were kept until June 1996 are shown in Table 2. Males started calling in May 1992 and eggs were laid a month later. Both sexes in this group of *C. romeri* matured in less than a year in captivity. Over 38% of the individuals in the study group survived until they were released to the wild almost 5 years after they had metamorphosed. Similar longevity was recorded at MZ. However, the ♀♀ monitored in this study stopped laying eggs after two to three breeding seasons, suggesting that the reproductively active life was much shorter.

In the summer of 1992, frogs in two terrariums, one with adults and the other with juveniles, exhibited signs of bacterial dermatosepticaemia, commonly known as the red-leg syndrome (Taylor *et al*., 2001). Within a week of the frogs first displaying symptoms, such as cutaneous haemorrhage on the hind limbs, nearly all the frogs in the two terrariums died. Substrates in the two terrariums were promptly changed and the tanks were disinfected. Holes were also drilled at the bottom of all the terrariums so that surplus water and wastes could drain properly. There were no further disease outbreaks after this modification. Histopathology did not determine the causative agent of this mortality event.

	MEAN	SD	RANGE	*N*
Clutch size	78	23 · 6	41–127	42
Fertilization rate (%)	98 · 9	2 · 1	89 · 0–100	42
Egg mortality rate (%)	7 · 5	10 · 5	0–61 · 2	42
Hatching time (days)			3–8	42
Tadpole mortality rate (%)	36 · 2	23 · 1	3 · 5–93 · 9	42
Average larval period (days)	46 · 9	12 · 6	28 · 6–83 · 3	36

Table 1. Clutch size, fertilization rate, mortality rates and larval duration of captive-bred eggs and tadpoles of Romer's tree frog *Chirixalus romeri* at the University of Hong Kong.

MZ At MZ, ♂♂ first called on the day after the frog's arrival in late April and continued intermittently until late May. Spawn was first deposited in two of the tanks on 1 May; 140 eggs were deposited in one tank and 20 eggs in the other. Five further clutches were laid in May. The size of clutches, eggs, newly hatched larvae and metamorphling frogs were recorded for this period (Table 3). The water temperature at which eggs hatched and

DATE	NO. ♂♂	NO. ♀♀	NO. EGG CLUTCHES	MORTALITY (♂.♀)	ANNUAL MORTALITY (%)
Year 1					
Aug 1991–Jul 1992	8	5	5		0
Year 2					
Aug 1992–Jul 1993	8	5	12	1.1	15 · 4
Year 3					
Aug 1993–Jul 1994	7	4	5	2.1	27 · 3
Year 4					
Aug 1994–Jul 1995	5	3	0	0.1	12 · 5
Year 5					
Aug 1995–Jun 1996	5	2	0	1.1	28 · 6

Table 2. Longevity, mortality and reproduction (number of egg clutches) of a group of Romer's tree frogs *Chirixalus romeri* maintained at the University of Hong Kong from August 1991 to June 1996.

	MEAN	RANGE	*N*
Clutch size	145	100–270	5
Egg size (mm)	1·5	1·0–2·0	
Hatching time (days)		3–4	
Larval length (mm)		5	
Average larval period (days)	50	40–60	36
Metamorphling length (mm)	8·4	8·0–8·5	30

Table 3. Clutch size, egg size, hatching time, larval length, larval period (metamorphosis) and metamorphling length of captive-bred eggs and tadpoles of Romer's tree frog *Chirixalus romeri* at Melbourne Zoo from May to December 1992.

larvae developed was 21–25 °C. Tadpole hind legs started to develop after 30 days at 21–25 °C, and metamorphosis was completed after 40–60 days. From this first breeding period, 250 froglets metamorphosed.

The young frogs grew steadily, but by mid-August 1992, at 1–4 months of age and 7–11 mm in body length, only 70 remained alive. The remainder had succumbed to a possible combination of dryness (overcome by retaining a small pile of moist sphagnum moss at one end of each tank) and their size. Being so small, they were very susceptible to environmental changes (build-up of wastes, temperature fluctuations, etc.) and it was not possible for the keepers to provide the high level of attention to respond rapidly enough to any deterioration in the frog's environment. At that time ten of the original 30 frogs remained. A further 27 young frogs died over the subsequent 4 months. Before death, the frogs exhibited a mucous build-up on the skin, twitching limbs and lack of limb co-ordination.

On 14 November 1992, at 4–5 months of age and 12–14 mm body length, the young ♂♂ (F1) started calling. This continued over the subsequent 2 months, from both the young ♂♂ and the remaining original ♂♂. Nineteen of the frogs that had metamorphosed 4–5 months earlier were placed together on 3 December 1992 and the first spawn for that season was deposited on 22 December. On that day, following days of rain and storms, about 140 infertile eggs and 100 fertile eggs were laid by the original ♀♀, and 120 eggs were laid by one of the F1 ♀♀. Of the fertile eggs, 94 and 116 tadpoles, respectively, hatched after 3–4 days. By the end of February 1993, 18 clutches of eggs had been recorded, seven from the original group (average 162 eggs, range 73–250; $n = 7$) and 11 from the F1s (average 72 eggs, range 20–108; $n = 11$). There was no difference in time to metamorphosis between tadpoles from the original frogs and those from the F1s. Hind legs started to appear at 25–30 days and front legs at 30–35 days, at temperatures between 21 and 30 °C. Metamorphosis was complete after 35–55 days, and on 1 May 1993, 530 froglets had metamorphosed. Their mean body length at metamorphosis was 6.2 mm (range 5.2–7.0; $n = 27$).

The young frogs were set up in groups of 10–20 in the 23 litre plastic tanks. Most of the F2 progeny were subsequently moved into a single large, mesh-topped enclosure measuring 1.8 m × 0.9 m × 0.6 m deep. This was landscaped with small grass tussocks and dry leaves, and had substrate depth and composition similar to the re-landscaped holding tanks. There was no plastic egg-crate mesh, however, but a sloped enclosure floor and a drainage outlet compensated for this.

Public display and education at MZ

Once the original group of frogs had adjusted to conditions at MZ and breeding had commenced, a public exhibit was established in the Zoo's Reptile House. Two plastic display enclosures, containing 30 frogs in total, were placed in the central off-exhibit area of the Reptile House, behind a large viewing window. Panels of information and photographs informed Zoo visitors about the breeding and conservation programme.

The programme was also used as a case study in wildlife conservation by the Zoo's Education Service in its teaching programmes for school students. These activities included behind-the-scenes visits with keepers, and were very popular with students and teachers.

Return and release of frogs

One of the main reasons for the captive-breeding programme at HKU and MZ was to establish secure populations of *C. romeri* in the wild following careful identification and assessment of appropriate sites. This component of the overall programme has subsequently proceeded and will be addressed in a separate publication (see also Dudgeon & Lau, 1999). Suffice to say that frogs bred at HKU and MZ have been crucial to the success of the reintroduction programme.

DISCUSSION

The reproductive behaviour of captive frogs matched that of wild individuals (Lau, 1998). Eggs were laid every month, although there were far more clutches deposited from February to August than between September and January. This differed from the field data, which indicated that *C. romeri* did not breed in the dry, cold months of October to January. The less severe conditions in captivity (temperature, relative humidity and food) and the constant availability of breeding sites probably allowed for continuous breeding by captive *C. romeri*. Breeding activity would appear to have been restricted by climatic conditions in nature and not by the endogenous state of the frogs. Indeed, calling ♂♂, tadpoles and eggs were present on a cold (around 15 °C) damp night in late February on Lantau Island. On another occasion in early March, calling ♂♂ and gravid ♀♀ were found on Chek Lap Kok Island on a rainy night at an air temperature of 14 °C.

Life-history data on wild *C. romeri* are lacking but a gravid ♀ from Chek Lap Kok Island laid 91 unfertilized eggs shortly after capture, which was comparable to the clutch size produced at HKU. The mean clutch was higher at MZ, but the possibility of this arising from two or more ♀♀ laying eggs in the same night in the one container cannot be ruled out.

At MZ, the average clutch size was greater from the original, wild-caught frogs than from the F1s, which is possibly linked to the original frogs being larger than the F1s at the time of spawning. Furthermore, the F1 frogs were larger than the F2s at metamorphosis. These findings could have resulted from a range of factors, including temperature, diet and density. Crowding in larval amphibians may stunt growth (Sokol, 1984; Travis, 1984; Woodward, 1987; Voigt, 1991; Tejedo & Reques, 1992; Goater, 1994) and diminish survival (Brockelman, 1969). Poor water quality is also known to decrease growth (Voigt, 1991) and water was therefore changed daily at both localities using aged tap water. Water straight from the tap contains chlorine and resulted in tadpole deaths at MZ before this practice was altered.

Hatching time at MZ was 2–4 days, compared with 3–8 days at HKU. The difference reflects the greater variation of water temperature experienced at HKU, which slowed hatching during the cooler months of the year. A negative correlation between temperature and developmental time has been recorded for many amphibian embryos (e.g. Whitaker, 1971; Duellman & Trueb, 1986). Tadpole mortality averaged 36.1% at HKU, and the mean larval duration was 47 days at HKU and 50 days at MZ. The fecundity and development data for captive *C. romeri* were within the range of anuran species having aquatic eggs and larvae (Duellman & Trueb, 1986). However, per cent mortality of eggs and, in particular, that of tadpoles at HKU was lower than the values reported for other species in nature [more than 50% egg mortality (Heyer, 1973) and over 90% tadpole mortality (Licht, 1974)]. The lack of predators and a stable laboratory environment were obvious contributing factors to this difference.

The data from HKU showed zero mortality over the first year after metamorphosis, increasing to 15.4% in the second year, 27.3% in the third year, 12.5% in the fourth year and 28.6% in the last 11 months monitored. This aspect, in conjunction with a capacity to mature and breed within 1 year, suggested that the life expectancy for *C. romeri* was 3–4 years. However, frogs captured as adults on Chek Lap Kok Island and subsequently held at MZ for over 3 years, coupled with frogs known to be almost 5 years of age when

released by HKU, point to a lifespan of at least 5 years for this species. The absence of predators and a stable laboratory environment may be factors enabling a longer lifespan in captivity. However, the ♀♀ monitored in the HKU study stopped laying eggs after two to three breeding seasons, suggesting that the reproductively active life was much shorter. Discrepancies between longevity records in captivity and life expectancy in the wild have been documented in other anurans (see Duellman & Trueb, 1986). In the case of *C. romeri*, the number of years that they were capable of reproduction probably gave more realistic estimates of life expectancy in nature; that is, they probably live for about 3 years in the wild. It is also possible that inadequate exposure to ultraviolet light could have affected lifespan. Hypovitaminosis D is generally thought to result from inadequate ultraviolet light exposure and can lead to metabolic bone disease in anurans, as was found in some of the *C. romeri* at MZ (Townsend & Cole, 1985; Allen & Oftedal, 1989).

The juvenile mortality rate was quite high (mean 49%) across all the studied groups of *C. romeri* at HKU, but this seems typical of most anurans (Duellman & Trueb, 1986). Similarly, the rapid growth to maturity seen in these *C. romeri* is also typical of many tropical anurans (Duellman & Trueb, 1986).

Chirixalus romeri turned out to be an ideal candidate for captive propagation. Its diminutive size and ability to breed in very small water bodies meant that many breeding groups could be kept in indoor terrariums. Although the clutch size is small relative to other amphibians, *C. romeri* is actually quite prolific because of the fast maturation rate (less than a year) and multiple clutches produced each season. Indeed, the captive-breeding programmes were so successful both at MZ and HKU that it was sometimes difficult to provide all the time and space necessary for the optimal care of so many frogs. The best approach, as was adopted in this joint project, is to seek co-operation from the international zoo community, which has the expertise for establishing captive-breeding programmes. Assistance from a number of zoological parks will also ensure that the future of a species/population does not depend on a single institution. The relevance of this approach was highlighted in this case when the outbreak of what may have been the red-leg syndrome caused mass mortality among frogs at HKU, and predation by ants killed many juveniles at MZ in a single night.

This species also offered good opportunities to deliver important key messages about active conservation of a threatened species in a zoo setting (at MZ), including the partnership between an Australian zoo (MZ) and an academic institution (HKU), supported by a private organization in Hong Kong (RHKJCCL). Such partnerships will help address the recognized deficit in such displays among the world's zoos (Peterson, 1996) and will play an increasingly important role in meeting the urgent conservation needs of many amphibians highlighted by the outcomes of the Global Amphibian Assessment (Stuart *et al*., 2004; see also Furrer & Corredor, in press; McGregor Reid & Zippel, in press).

The *C. romeri* breeding release programme is the first such documented case for a rare or threatened tropical anuran. The high initial success rate in this instance was owing to an understanding of the requirements of the species through scientific research, the presence of suitable, unoccupied habitats at the release sites and the biology of *C. romeri* (including an ability to breed in very small man-made water bodies).

The project was a watershed in MZ's conservation activities in Asia, as it was the Zoo's first instance of direct *ex situ* involvement and generated considerable media interest. However, there are two issues that warrant further comment:

First, the lack of conservation listing for this species at the beginning of the programme made the MZ involvement so much easier than it might otherwise have been. Indeed, it was only officially listed under the Wild Animals Protection Ordinance in Hong Kong on 4 June 1992. If it had been listed by CITES (Convention on International Trade of Endangered Species of Wild Fauna and Flora) or the IUCN (The World Conservation

Union), it would most likely have taken longer to obtain import approval into Australia, with potential implications for the subsequent success of the breeding programme. As it was, importation of *C. romeri* into Australia required Vertebrate Pests Committee recategorization, as the species was not maintained in Australia when the request for conservation assistance was received in 1991. The frogs to be imported into Australia were transported in a plane's passenger compartment, necessitating special dispensation from the Australian Civil Aviation Authority (Banks, 1996).

Second, *C. romeri* is a tiny, drab-brown frog with no known commercial value, living in densely populated Hong Kong. Through sympathetic media coverage, good communication and support from well-known organizations, such as the RHKJCCL, this frog became one of the best-known wild creatures in Hong Kong. The potential of using amphibians to increase public awareness about the conservation of natural environments should be explored whenever appropriate.

The involvement of both HKU and MZ with *C. romeri* was driven by a need to support the sustainable conservation of this species. In order to achieve this goal, conservation-breeding programmes must maintain natural adaptations (Kaumanns, 1996) and avoid artificial selection (Kleiman, 1980; Allendorf, 1993; Beebee, 1996) because the resultant characteristics may be disadvantageous in the natural environment (Cover *et al*., 1994). Harmful genetic changes can also occur in captivity through genetic drift and inbreeding, and will influence the chances of re-establishing wild populations (Allendorf, 1993; Mallinson, 1995; Beebee, 1996). Captive frogs should be maintained in breeding groups to allow normal interactions between ♂♂, and sexual selection by ♀♀. However, this means that a detailed pedigree of individuals is difficult to obtain and controlled matings are often impossible to achieve. Because of their high individual fecundity, rapid selection of frogs during captivity is possible (Allendorf, 1993). For this reason, captive breeding should be used as a measure only for as long as it is required to address key components of a broader conservation programme for a threatened species.

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PRODUCTS MENTIONED IN THE TEXT

Berlese–Tullgren funnel: plastic funnel for separating small ground arthropods from soil, manufactured by Griffin Scientific as a branch of Thermo Fisher Scientific, Griffin Education, Bishop Meadow Road, Loughborough LE11 5RG, UK. http://www.griffineducation.co.uk/

NEC fluorescent lights: lighting, supplied by Nelson Industries, 4 Forbes Close, Knoxfield, Vic. 3180, Australia. http://www.nelsonlamps.com.au/

Sanyu tropical fish food: fish diet, distributed by American Aquarium Products, Grants Pass, OR 97526, USA. www.americanaquariumproducts.com

Sylvania black-light fluorescent tubes: light bulbs, manufactured by Sylvania Lighting Australasia Pty Ltd, Riverview Business Park, 87 Moreland Street, Footscray, Vic., 3011, Australia. http://www.sla.net.au/

Tetra: vitamin mix, manufactured by Tetra Werke, Herrenteich 78, Melle 49324, Germany. www.tetra-fish.-com/TetraFish.home

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